

AD _____

GRANT NUMBER DAMD17-97-1-7108

TITLE: CEA, PSA and Other Biomarkers in Nipple Fluid for Early
Cancer Detection

PRINCIPAL INVESTIGATOR: Frederick P. Li, M.D.

CONTRACTING ORGANIZATION: Dana Farber Cancer Institute
Boston, Massachusetts 02115-6084

REPORT DATE: June 1998

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

BEST QUALITY IMAGED **4**

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY <i>(Leave blank)</i>			2. REPORT DATE June 1998	3. REPORT TYPE AND DATES COVERED Annual (1 Jun 97 - 31 May 98)	
4. TITLE AND SUBTITLE CEA, PSA and Other Biomarkers in Nipple Fluid for Early Cancer Detection			5. FUNDING NUMBERS DAMD17-97-1-7108		
6. AUTHOR(S) Frederick P. Li, M.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Dana Farber Cancer Institute Boston, Massachusetts 02115-6084			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER		
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE		
13. ABSTRACT <i>(Maximum 200 words)</i> This proposal will attempt to develop nipple fluid-based test(s) for early breast cancer detection. If successful, these non-invasive breast nipple fluid studies could complement mammography, particularly for women under age 50. Our studies in breast nipple fluid have examined 2 tumor biomarkers, carcinoembryonic antigen and prostate-specific antigen (CEA and PSA). In clinically cancer-free women, CEA titers vary widely in 215 breast nipple fluid samples. The median CEA is 1,100 ng/ml, >200-fold higher than normal serum levels. Likewise, PSA median level in 148 nipple fluids is 55 ng/ml, when serum PSA is virtually 0 in women. High CEA and PSA levels in nipple fluid are new and unexpected findings. Our IDEA study will determine whether CEA and PSA levels are biomarkers for breast cancer. Nipple fluid CEA and PSA titers from 75 women with untreated breast cancer will be examined and compared with titers of 150 cancer-free, low risk women. Collection of nipple fluids from pre-operative breast cancer patients is labor intensive. As new breast cancer biomarkers such as telomerase activity and assays for cancer-associated nuclear matrix proteins are developed, their levels can be rapidly studied in the nipple fluid specimens collected and banked through this study.					
14. SUBJECT TERMS Breast Cancer nipple fluid			15. NUMBER OF PAGES 12		
			16. PRICE CODE		
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited		

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Edith Phi 6/1961

TABLE OF CONTENTS

	Page
Front Cover.....	1
Report Documentation Page.....	2
Foreword.....	3
Table of Contents.....	4
Introduction.....	5
Body.....	5
Conclusions.....	8
References.....	9
Figure 1.....	11
Figure 2.....	12

INTRODUCTION: Breast cancer is the most common cancer in American women. Cure rates for breast cancer are highly correlated with early disease stage at initial diagnosis. Recent decline in breast cancer mortality rates in the U.S. and elsewhere is likely due to the benefits of both early detection and more effective treatments. Mammography is an established screening modality that has been repeatedly shown to reduce breast cancer mortality in women age 50 and over. Although efficacy of mammographic screening for women under age 50 remains controversial, recent data from the Swedish 2-county study indicates a reduction in breast cancer mortality among these younger screenees. Clinical breast exam and breast self-examination are additional methods of early detection.

In this IDEA project, we are seeking to complement mammography and physical exams with novel breast cancer detection methods based on nipple fluid analyses. We will determine whether carcinoembryonic antigen (CEA) and prostate specific antigen (PSA) in breast nipple fluid can be used as biomarkers of early breast cancer. Nipple fluid can be obtained in approximately 50 percent of all American women, or 50 million potential beneficiaries of a validated test if found. Nipple fluid CEA/PSA studies offer several attractive features. Firstly, the methods of specimen collection, developed nearly 50 years ago, are safe and non-invasive. Also, standard laboratory assays for CEA and PSA are available and inexpensive. Although CEA and PSA in nipple fluid have not been examined previously, elevated CEA titers in spontaneous pathologic breast nipple discharges are highly predictive of breast cancer. Lastly, other new tumor markers can be sought in the cellular and liquid fractions of breast fluid.

Our previous work on cancer-free women have found a wide range of CEA and PSA titers in nipple fluid (median CEA, 1,087 ng/ml; and median PSA, 55 ng/ml). In this project, we will examine whether CEA/PSA titers are higher in nipple fluids from pre-operative cancer-bearing breasts, as compared with fluids from normal breasts. Nipple fluid analyses can be extended in future studies to identify new candidate biomarkers for cancer, such as telomerase activity and cancer-associated nuclear matrix proteins.

BODY:

Experimental Methods. Nearly 50 years ago Papanicolaou, the developer of the Pap smear for cervical cancer, pioneered the use of a breast pump to obtain nipple fluid for cytologic analysis for breast cancer (1). A limitation to nipple fluid analyses has been the small quantity of the material. Approximately 10-100ul of nipple fluid is obtainable from 30-60% of American women. The fluid is more readily obtained from women under age 50, for whom mammography may provide benefit (2-4). Breast cancers have been diagnosed by nipple fluid cytology in anecdotal published reports (1, 5, 6). Thus, there are presently 50 million potential candidates for nipple fluid exams in the U.S., if validated early cancer detection tests were to become available (7, 8).

Petrakis has analyzed nipple fluid collected from nearly 50,000 women in recent decades (7, 8). In a prospective study of 2,701 women with nearly 30,000 person-years of follow-up, subjects whose nipple fluid showed cytologic atypia had an increased risk of subsequent breast cancer (relative risk=4.9; 95%CI, 1.7-13.9) (9). However, cytologic diagnosis is limited by the presence of only 100 breast epithelial cells in the typical nipple aspirate specimen, and the Petrakis results are unconfirmed (5, 8, 9). Investigators have also found that the liquid fraction of nipple fluid contains high levels of various steroid hormones, proteins, lipids, and growth factors (8, 10, 11) but not tumor biomarkers indicative of early breast cancers.

Assumptions. Our hypothesis is that CEA and PSA in breast nipple fluids are early markers of breast cancer. These 2 tumor biomarkers have been widely studied in peripheral blood using standard assays. CEA is an oncofetal antigen that is detected in 50-90% of primary breast tumors using immunohistochemical

techniques. CEA is often elevated in serum of patients with metastatic breast cancer, and is widely used to monitor response to cancer treatment (12). Recent studies in Japan of abnormal spontaneous breast discharge (not nipple fluid collected by suction) have found elevated CEA levels among women whose workup revealed a previously unrecognized breast cancer. Inaji et al. reported using a different assay that showed CEA levels in spontaneous abnormal nipple discharge exceeded 600 ng/ml in 6 of 7 women with nonpalpable early breast cancers (tumor cells were later shown to express CEA), and 0 of 23 with non-cancerous breast discharges (13). Three additional reports from Japan have confirmed the finding (14-16). In one, high nipple discharge CEA titers had a sensitivity of 76% and specificity of 79% for marking the presence of breast cancer (16). Additionally, prostate-specific antigen (PSA) was thought to be virtually absent in women (17). Serum PSA levels are low (up to 4 ng/ml) in healthy men, and elevated serum PSA levels are clinically used as a marker for prostate cancer. Serum PSA is undetectable in healthy women. However, recent reports have identified high PSA levels in both extracts of female breast cancers and in cell culture media of PSA-positive breast tumor lines (17-21). Our primary objective of this project is to initiate the multistep process of examining nipple fluid CEA and PSA as candidate biomarkers for the neoplasm.

Procedures. We have procedures to collect nipple fluids and risk factor data by questionnaire from 75 cases before surgery/therapy for breast cancer, and 150 cancer-free controls. Using standard assays, PSA and CEA will be assayed to determine whether these established tumor markers are significantly higher in nipple fluids from cancer cases as compared with controls. We also seek additional tumor biomarkers in the cellular and liquid fractions of nipple fluid that might identify early breast cancer. Using a case-control study design, CEA and PSA (as well as other candidate tumor biomarkers) will be compared in 75 pre-treatment breast cancer patients (cases) and 150 women without cancer (controls). Controls will be frequency-matched to cases on 2 strata of age (<50 years, and 50 or older) and a 2:1 ratio of controls to cases. Controls will be limited to cancer-free women over age 40 with no history of cancer or pre-cancerous breast tumors. Sample size is limited by the cost of identifying and enrolling untreated breast cancer patients in the short interval between cancer diagnosis and surgery. During this period, patients are anxious and we take the time to be supportive and sensitive to their distress.

We have obtained IRB approval to collect and analyze breast nipple fluid samples from women through 4 Boston hospitals that register 2000 breast cancer cases annually. These are Dana-Farber Cancer Institute, Brigham and Women's Hospital, Beth Israel Hospital and Faulkner Hospital. Previously, specimens have been collected primarily from women presenting for routine screening mammograms; few had breast cancer personally. Samples are processed and analysed under supervision of the PI in the Molecular Diagnostics Laboratory of Dana Farber Cancer Institute. All proposed collection methods have been pre-tested in our preliminary studies.

With signed consent from an eligible woman, the breast nipple is gently cleansed. She is asked to gently compress her breast. The suction cup is placed over the nipple, and suction is gently applied for 5-10 seconds using a 20 ml syringe. The procedure is less uncomfortable and less intrusive than breast compression for mammograms. If a droplet appears, the fluid is collected into a microcentrifuge tube and the process is repeated on the opposite breast. The residual moisture on the nipple is spread onto a slide and stored for future cytologic studies. The fluid is centrifuged, and the cell pellet and supernatent are separately aliquoted for storage at -70C. No major complications from the procedure have been encountered in 1000 collection attempts. The procedure itself takes about 5 minutes and is well tolerated. Finding potential participants, explaining the procedure, obtaining informed consent, and collection of basic risk factor data take more than 30 minutes. Nipple fluid collection for research purposes, when performed with sensitivity and respect for the participant, is a labor intensive process. The nipple fluid samples are examined for CEA using the commercial immunoenzymometric assay kit, AIA-PACK CEA (Tosoh Medics, Foster City, CA).

Results. We have examined in pilot studies a total of 215 nipple fluids from 147 women; 21 samples were re-tested and showed highly reproducible results. Right and left breast fluid samples for the same subject consistently yielded comparable CEA titers (Spearman rank correlation=0.78, p<0.01). The median CEA value was 1100 ng/ml (range, undetectable to 8,400 ng/ml). The 4 specimens from women with pre-operative breast cancers showed a median titer of 2000 ng/ml, nearly double that for cancer-free control subjects. Samples were also tested by Western blot using a mouse anti-human monoclonal antibody (IgG1; Piece, Rockford, Illinois) along with controls (purified human CEA protein; Calbiochem Novabiochem, San Diego, CA). These nipple fluids contained the predicted 180 kD CEA glycoprotein. CEA in suctioned nipple fluid had never been reported previously (22).

Table 1 summarizes the samples collected to date. Among over 1,200 attempts, 560 samples were collected. Of these, 52 had breasts that contained carcinoma. In addition, 37 had atypical ductal hyperplasia, 17 had ductal carcinoma *in situ*, and 2 had lobular carcinoma *in situ* at the time of collection. These diagnoses were confirmed by histological examination of the tumor specimen after excision. Samples are still being collected.

Table 1. Nipple Fluid Collected, by patient diagnosis

Diagnosis	# collected
Carcinoma	52
DCIS	17
LCIS	2
ADH	37
No Breast Tumors	452+
TOTAL	560

+Most normal samples collected prior to DOD study

To date, we have a total of 108 nipple fluids from invasive breast carcinoma or precancerous breast lesions. We have resisted the temptation to study our major endpoint, comparison of CEA and PSA in nipple fluids of tumor breasts and normal breasts. The reason is that our quantities of nipple fluids are minute, and results may fluctuate slightly with different assay kits employed over time. Instead, we will finish our collection for the study and perform all laboratory analyses at one time to standardize all assay conditions.

In the meantime, we have explored other novel assays. In collaboration with Dr. Jerry Shay at MD Anderson Hospital, we have begun to look at telomerase levels in nipple fluids. The original assay used did not have adequate sensitivity to detect telomerase in our small quantities of material. Dr. Shay has since developed a more sensitive assay, and additional biospecimens have been sent to him.

A second collaboration with Dr. Jose Costa and Paul Lizardi at Yale uses a beta helicase PCR-based assay that they have developed (23). In five nipple fluid samples analyzed to date, exons 5, 7 and 8 of the p53 gene have been successfully amplified in several samples; SSCP (single strand confirmation polymorphism) analysis of one sample indicates wildtype alleles (Table 2). Interestingly, in sample 3, exons 5 and 8 were successfully amplified whereas exon 7 was not. This finding may be due to technical problems.

Alternatively, exon 7 may have been deleted in the sample, and additional studies are in progress. Their technique of whole gene amplification (WGA) obtained PCR products and *k-ras*, and the three exons of p53 were successfully amplified in one of these samples (Table 2). We have recently sent these collaborators the residual pellets after removal of the aqueous portion of nipple fluid aspirate samples. These pellets are known to contain cells, whereas work is in progress in Dr. Lizardi's lab to amplify DNA with these pellets derived from nipple aspirate fluid.

TABLE 2. SUMMARY OF DATA COLLECTED (5/29/98)

#	sample	DNA isolation	DNA yield	Genomic DNA								WGA product	WGA product		
				K-ras	p53	p53	p53	SSCP	SSCP	WGA	K-ras		p53	p53	p53
ex 1	ex 5	ex 7	ex 8	p53 ex 7	p53 ex 8	product	ex 1	ex 5	ex 7	ex 8					
1	DF002796-R	Na I	1-2ng		no	no	no				no				
2	DF001481-R	Na I	1-2ng		yes	yes	yes	wild type	wild type		no				
3	DF000455-R	GeneReleaser	not measured	yes	no	yes					yes				
4	DF001703-L	GeneReleaser	not measured	yes	yes	yes					yes				
5	DF001715-R	GeneReleaser	not measured	yes	yes	yes					yes	yes	yes	yes	yes

A third collaboration is with Matriotech, a biotechnology firm that has focused their diagnostic development on nuclear matrix proteins (24, 25). By polyacrylamide gel electrophoresis (PAGE), known proteins such as albumin were found in similar levels in all specimens, confirming our prior data on albumin in nipple fluids independently determined by caloriometric reactions with bromophenol blue (Sigma Diagnostics, St. Louis, MO) (Figure 1). Additionally, various samples showed differing patterns of concentration and distribution of other proteins yet to be identified. Additional analysis focused on a breast cancer associated nuclear matrix protein developed by Matriotech. In 28 specimens sent to them, this nuclear matrix protein was found to be expressed in different levels among nipple fluid specimens (Figure 2). Isolated observations suggest that elevated levels may be associated with fibrocystic diseases of the breast, but additional studies are underway.

Discussion. We have little definitive results to report because the work is still in progress. We are continuing to accrue specimens from preoperative breast cancer patients, despite the challenges to obtaining these precious specimens. Recently, one of the participating institutions, Faulkner Hospital, is undergoing new construction in its mammography suites. Fluid collection at that site has been discontinued at least temporarily. We expect to meet our target goals by the end of this grant, and particularly, the primary aim of the study to compare CEA and PSA in cancerous nipple fluids and normals.

CONCLUSIONS: We are studying small quantities of nipple fluids, and will assay for CEA and PSA after completion of specimen collection. The telomerase assays have not been informative to date. On the other hand, a nuclear matrix protein has been found at different levels in nipple fluids, and we need to find those most useful for identifying breast cancer. A new and promising development is the ability to PCR-amplify known breast cancer-associated genes in nipple fluids. The method may prove useful in finding these cancer-associated mutations in affected breasts, so early detection can be improved.

REFERENCES:

1. Papanicolaou, G. N., Holmquist, D. G., Bader, G. M., and Falk, E. A. Exfoliate cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases of the breast. *Cancer*, 2: 377-409, 1958.
2. Fletcher, S. W., Black, W., Harris, R., and al, e. Report of the International Workshop on Screening for Breast Cancer. *Journal of the National Cancer Institute*, 85: 1644-1656, 1993.
3. Sickles, E. A., and Kopans, D. B. Mammographic screening for women aged 40 to 49 years: the primary care practitioner's dilemma. *Annals of Internal Medicine*, 122: 534-538, 1995.
4. Kerlikowske, K., Grady, D., Rubin, S. M., Sandrock, C., and Ernster, V. L. Efficacy of screening mammography. *Journal of the American Medical Association*, 273: 149-154, 1995.
5. Sartorius, O. W., Smith, H. S., Morris, P., Benedict, D., and Friesen, L. Cytologic evaluation of breast fluid in the detection of breast disease. *Journal of the National Cancer Institute*, 59: 1073-1080, 1977.
6. King, E. B., Barrett, D., and Petrakis, N. L. Cellular composition of the nipple aspirate specimen of breast fluid. II. Abnormal findings. *American Journal of Clinical Pathology*, 64: 739-748, 1985.
7. Petrakis, N. L., Mason, L., Lee, R., Sugimoto, B., Pawson, S., and Catchpool, F. Association of race, age, menopausal status, and cerumen type with breast fluid secretion in nonlactating women, as determined by nipple aspiration. *Journal of the National Cancer Institute*, 54: 829-834, 1975.
8. Petrakis, N. L. Physiologic, biochemical and cytologic aspects of nipple aspirate fluid. *Breast Cancer Research and Treatment*, 8: 7-19, 1986.
9. Wrensch, M. R., Petrakis, N. L., King, E. B., Miike, R., Mason, L., and Chew, K. L. Breast cancer incidence in women with abnormal cytology in nipple aspirates of breast fluid. *American Journal of Epidemiology*, 135: 130-141, 1992.
10. Rose, D. P. Diet, serum, breast fluid growth hormone and prolactin levels in normal premenopausal Finnish and American women. *Nutrition and Cancer*, 11: 179-187, 1988.
11. Rose, D. P. Hormones and growth factors in nipple aspirates from normal women and benign breast disease patients. *Cancer Detection and Prevention*, 16: 43-51, 1992.
12. vonKleist, S., Wittekind, C., Sandritter, W., and Gropp, H. CEA positivity in sera and breast tumor tissues obtained from the same patients. *Pathology, Research and Practice*, 173: 390-401, 1982.
13. Inaji, H., Yayoi, E., Maeura, Y., Matsuura, N., Tominaga, S., Koyama, H., Takatsuka, Y., and Mori, T. Carcinoembryonic antigen estimation in nipple discharge as an adjunctive tool in the diagnosis of early breast cancer. *Cancer*, 60: 3008-3013, 1987.

14. Inaji, H., Koyama, H., Motomura, K., Noguchi, S., Mori, Y., Kimura, Y., Sugano, K., and Ohkura, H. ErbB-2 protein levels in nipple discharge: role in diagnosis of early breast cancer. *Tumor Biology*, 14: 271-278, 1993.

15. Yayoi, E., Furukawa, J., Sekimoto, M., Tateishi, H., Kinuta, M., Maruyama, H., and Okamura, J. Early detection of non-palpable (TO) breast cancer: the diagnostic procedure for pathological discharge from the nipple. *Jpn J Cancer & Chemotherapy*, 2: 133-139, 1994.

16. Mori, T., Inaji, H., Koyama, H., Abe, R., Nihei, M., Izuo, M., Ogawa, T., Enomoto, K., Sato, H., Kasumi, F., Sakamoto, G., Makita, M., Miura, S., Kojima, O., Miyauchi, K., Wada, A., Yayoi, E., Takatsuka, Y., Morimoto, T., Komaki, K., Miyauchi, Y., Sato, M., and Kudo, T. Evaluation of an improved dot-immunobinding assay for carcinoembryonic antigen determination in nipple discharge in early breast cancer: results of a multicenter study. *Japanese Journal of Clinical Oncology*, 22: 371-376, 1992.

17. Diamandis, E. P., Yu, H., and Sutherland, D. J. A. Detection of prostate-specific antigen immunoreactivity in breast tumors. *Breast Cancer Research and Treatment*, 32: 301-310, 1994.

18. Monne, M., Croce, C. M., Yu, H., and Diamandis, E. P. Molecular characterization of prostate-specific antigen messenger RNA expressed in breast tumors. *Cancer Research*, 54: 6344-6347, 1994.

19. Yu, H., Diamandis, E. P., and Sutherland, D. J. A. Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. *Clinical Biochemistry*, 27: 75-79, 1994.

20. Yu, H., Diamandis, E. P., Zarghami, N., and Grass, L. Induction of prostate specific antigen production by steroids and tamoxifen in breast cancer cell lines. *Breast Cancer Research and Treatment*, 32: 291-300, 1994.

21. Yu, H., and Diamandis, E. P. Prostate-specific antigen in milk of lactating women. *Clinical Chemistry*, 41(1): 54-58, 1995.

22. Foretova, L., Verselis, S., Andrade, A. F. F., Garber, J. E., and Li, F. P. Carcinoembryonic antigen (CEA) detection in nipple aspirate fluid (NAF) from women at high risk for breast cancer. *Proceedings of ASCO*, 14: 100, 1995.

23. Tyagi, S., Landegren, U., Tazi, M., Lizardi, P., and Kramer, F. Extremely sensitive, background-free gene detection using binary probes and beta replicase. *PNAS*, 93: 5395-5400, 1996.

24. Keesee, S., Briggman, J., Thill, G., and Wu, Y. Utilization of nuclear matrix proteins for cancer diagnosis. *Crit Rev Eukaryot Gene Expr*, 6: 189-214, 1996.

25. Keesee, S. K. Nuclear matrix proteins in human colon cancer. *Proceedings of the National Academy of Sciences USA*, 91: 1913-1916, 1994.

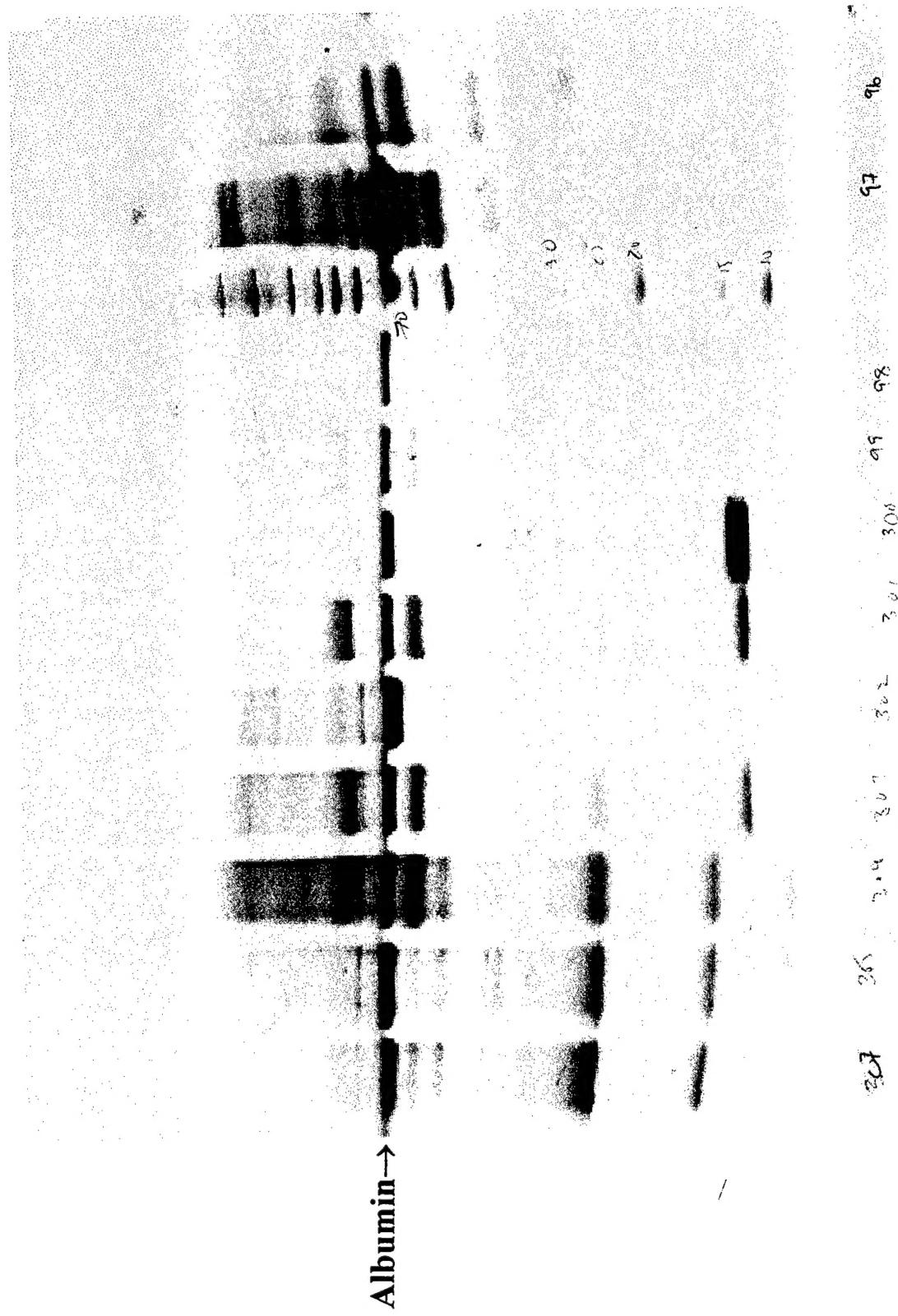


FIGURE 1. Nipple fluids always contain albumin, whereas other proteins of diverse sizes are present in markedly different titers.

FIGURE 2. Wide range of a breast cancer-associated nuclear matrix protein identified by our collaborator (Matritech) in nipple fluids. Titers above 7 ng/ml are considered high, and other studies are in progress.

